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WINTER WHEAT GRAIN YIELD RESPONSE TO FUNGICIDE APPLICATION AT DIFFERENT STAGES AND FUSARIUM HEAD BLIGHT IS RATHER INFLUENCED BY VARIETY AND YEAR

Valentina Spanic[1*], Katarina Sunic[1], Jurica Duvnjak[1], Jurislav Babic[2], Georg Drezner[1]

[1]Agricultural Institute Osijek, Osijek, Juzno predgradje 17, 31000 Osijek, Croatia
[2]Faculty of Food Technology Osijek, Josip Juraj Strossmayer University of Osijek, Franje Kuhaca 18, 31000 Osijek, Croatia
[*]Corresponding author. E-mail: valentina.spanic@poljinos.hr

ABSTRACT

Winter wheat is susceptible to several diseases throughout the vegetative season whereas fungicide treatments are protection used to combat fungal pathogens and to improve plant growth thus mitigating grain yield reductions. One of the main diseases is Fusarium head blight (FHB) which can be a huge problem in wheat production. Twelve winter wheat varieties varying in FHB sensitivity were tested for control of FHB using fungicide treatments made in tillering or/and heading stage, with or without inoculation with *Fusarium* spp. to determine the grain yield response to fungicide application at different growth stages. The grain yield from fungicide treated plots was compared to non-treated plots in two seeding rates and *Fusarium* inoculated plots in two growing seasons (2019/2020 and 2020/2021). The average area under the disease progress curve (AUDPC) for Type I resistance was 109.48 in 2020 and 99.33 in 2021 year in VI treatment where fungicide application in heading and *Fusarium* inoculation simultaneously were performed. In VII treatment where only *Fusarium* inoculation was applied, AUDPC for Type I resistance in 2020 was 371.88 in average, while in 2021 that was 199.18 in average. Wheat varieties treated with a fungicide in the heading stage at the first year of investigation (2020) had higher grain yield, compared to non-treated plots or treated in tillering stage. These results indicated that application of fungicides in heading stage when there is sufficient moisture in May and June may increase chances of profitability from fungicide application at that growth stage. Cumulative rainfall from January till May, at year 2021 had a positive effect on the grain yield, when accumulated rainfall in this period increased chances of getting a higher yield response from fungicide application in the tillering stage. Fungicide applications should always be implemented in combination with sound agronomic management and FHB resistant varieties.

Keywords: fungicide, Fusarium, weather conditions, wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important crops and is a basic source of carbohydrates and proteins for humans (Mickky et al., 2020). The negative trend of sensitivity of wheat varieties to environmental factors is especially visible in Europe (Schauberger et al., 2018). Amongst the various biotic factors affecting wheat productivity, diseases are one of the most important ones. However, changes in climatic conditions have increased disease contamination in wheat thus reducing grain yield and quality. Wheat diseases can develop any time environmental conditions are favorable for disease development. Particularly rainfall and temperature cause fungal disease epidemic development (Wiik and Ewaldz, 2009) whereas rainfall is the most important factor for diseases to develop (Thompson et al., 2014). Besides pathogen dispersal through rainfall, leaf and head wetness period for infection initiation can be influenced in that way (Rowlandson et al., 2015). Major component of control of diseases is through genetic resistance and/or fungicide application (Torriani et al., 2015; Pietrusińska and Tratwal, 2020). One of the most important diseases due to its yield-reducing ability is FHB (ElDoliefy et al., 2020). The environmental conditions at local level and soil management practices

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may drive FHB epidemics and increase mycotoxin concentration (Scala et al., 2016). Prolonged precipitation during anthesis will promote conditions for FHB infection (Parry et al., 1995; Kriss et al., 2010, Okorski et al., 2022). Usually successful FHB infections will occur when wetness period is of at least 24 hours and temperatures above 15°C (Parry et al., 1995). Even after flowering during grain filling warm and moist conditions increase the occurrence of FHB and mycotoxin contamination (Kriss et al., 2012). The best protection for wheat plants is usage of FHB resistant varieties (Buerstmayr et al., 1999; Beres et al., 2018). Furthermore, FHB losses may be decreased by the combination of FHB tolerant varieties, fungicides and agronomic practices (Dahl and Wilson, 2018). But however, control with crop rotation and fungicides are only slightly effective.

The objective of the present study was to estimate the most important economic trait, grain yield, of winter wheat by evaluating a set of eleven varieties adapted for production in Croatia and one foreign winter wheat variety in the presence of fungicide treatments at two growth stages separately and together, as well as in *Fusarium* inoculated treatment and in two treatments in two seeding rates in absence of any fungicide or *Fusarium* inoculation.

MATERIAL AND METHODS

Plant material, experimental layout and treatments

Twelve winter wheat varieties were sown at Osijek (45°32' N, 18°44' E) in 2019 and 2020 between October 10th and 20th where soil type is eutric cambisol. For each of two growing seasons, 2019/2020 and 2020/2021, the experimental design consisted of a split-plot design with main plots in randomized complete blocks of two replications. Plots measured 1.08 m wide by 7.00 m long. Each winter wheat variety was sown in one treatment (in total there was seven treatments). First two treatments (I and II) were grown without usage of fungicides by planting 330 and 600 g of seeds per m^2. Next three treatments (III, IV and V) were treated with fungicide at different growth stages (treated in tillering, in tillering and heading together, and in heading), while the last two treatments (VI and VII) were subjected to *Fusarium* inoculations, with VI treatment being protected with fungicide in the heading stage. Prior to planting, to control seed-borne diseases, the seed was treated with Maxim Extra 050 FS (fludioxonil 25 g L^{-1} + difenoconazole 25 g L^{-1}) at a rate of 125 mL 100 kg^{-1}. Seeding rate was 330 pure live seeds per square meter or 2.7 million seeds per hectare (except in II treatment). The previous crop in 2019/2020 was maize, while in 2020/2021 the precrop was soybean. Standard agronomic practices for fertilizer management [100 kg ha^{-1} carbamide (UREA) + 400 kg ha^{-1} 7:20:30 (nitrogen, phosphorus, potassium) at planting and 120 kg ha^{-1} of calcium ammonium nitrate (CAN) at green-up in spring] were applied. The weed control was conducted at both investigated years two times at BBCH 25 and 45 with Sekator OD (amidosulfuron 100 g L^{-1} + iodosulfuron 25 g L^{-1} + mefenpyr-diethyl 250 g L^{-1}) and Tomigan 250 EC (fluroxypyr 250 g L^{-1}), while pests were treated two times with Nurelle (chlorpyrifos 500 g L^{-1} + cypermethrin 50 g L^{-1}) and Vantex 60 CS (gamma-cyhalothrin 60 g L^{-1}) in 2019/2020, while in 2019/2020 one additional spraying with Vantex 60 CS was conducted. Fungicide used in tillering stage in 2020 was Ascra Xpro (prothioconazole 130 g L^{-1} + bixafen 65 g L^{-1} + fluopyram 65 g L^{-1}), and in 2021 Elatus Era (prothioconazole 130 g L^{-1} + benzvindiflopyr 75 g L^{-1}). The fungicide Prosaro 250 EC (prothioconazole 125 g L^{-1} + tebuconazole 125 g L^{-1}) was applied for the treatments V and VI in the stage of heading at a rate of 1L ha^{-1} at both investigated years.

The vegetative year 2019/2020 received the higher total rainfall (408.6 mm), compared to the second year of investigation (2020/2021) (548.8 mm). Temperatures were comparable across investigated two years during the critical wheat growing period around anthesis. But it is important to note that in May and June in 2020 the total rainfall was 126.8 mm, while in 2021 it was 1.64 fold lower (77.3 mm) (Figure 1).

In 2020 and 2021, in treatments VI and VII *Fusarium* inoculations were performed at the heading stage, after what each variety was visually evaluated for Type I resistance (initial resistance) for FHB at 10, 14, 18, 22 and 26 days after *Fusarium* inoculation. Plots were harvested at maturity using a Wintersteiger cereal plot combine-harvester at the beginning of July and seed weight from each plot was recorded and converted to yield (dt ha^{-1}) after adjusting for moisture content at 14%.

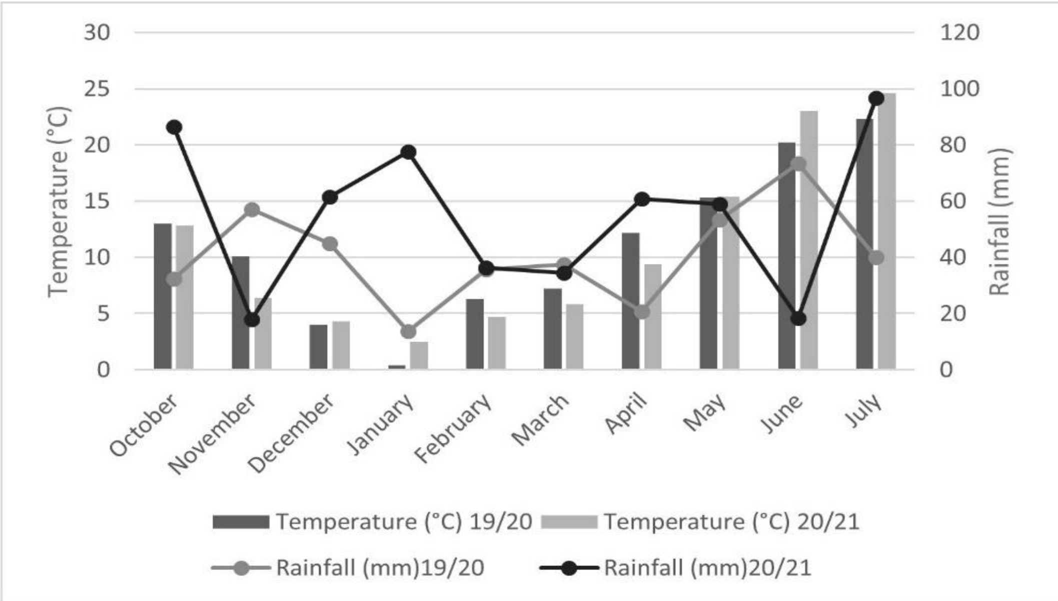

Figure 1. Climate diagram for investigated two years (2019/2020 and 2020/2021) of the study

Inoculum preparation and inoculation procedure

We selected one isolate of *F. culmorum* (IFA 104) and one isolate of *F. graminearum* (PIO 31). All isolates were maintained in permanent cultures at 4°C until production of inocula was performed. First of all, those two isolates were transferred from permanent cultures to synthetic nutrient-poor agar (SNA) in Petri dishes and incubated at room temperature for 14 days. Piece of SNA agar was further transferred to a mixture of wheat and oat grains (3:1 by volume). After two weeks' conidial suspension was calibrated using a hemocytometer (Bürker-Türk, Hecht Assistent) to reach 10×10^4 conidia mL^{-1}. At the heading stage (Zadok's scale 55-59) (Zadoks et al., 1974) in the treatments VI and VII, inoculum was sprayed on the plant heads with tractor-back sprayer (100 mL of inoculum per m^2) and repeated two days after. To set up infection misting was provided by spraying with a tractor back-sprayer few times in next 24 hours after inoculations.

FHB type I resistance

Initial resistance was calculated as the percentage of heads with symptoms (30 heads per plot were analysed), where the scores were converted into percentages of the heads exhibiting symptoms. The type I resistance was estimated on days 10, 14, 18, 22, and 26 after inoculation. Based on those five measurements, the area under the disease progress curve (AUDPC) for type I FHB resistance was calculated according to formula:

$$AUDPC = \sum_{i=1}^{n} \{ \left[\frac{Yi + Yi - 1}{2} \right] * (Xi - Xi - 1) \}$$

where

Yi is percentage of visibly infected spikelets (Yi/100) at the i^{th} observation, Xi is day of the ith observation and n is total number of observations.

Statistical analysis

An analysis of variance (ANOVA) was conducted for all years, treatments and varieties to isolate and evaluate the effect of the fungicide application/*Fusarium* inoculation on the grain yield. Statistica 12 software was used to perform ANOVA followed by Fisher LSD test to detect significant difference between means at a significance level of $p < 0.05$. To determine if the grain yield was statistically influenced as a response to fungicide application in different stages of plant growth or *Fusarium* inoculation in the heading, wheat varieties at each growing season were arranged in ascending order of the grain yield for different treatments. Results of the grain yield were expressed as mean value of two replications ± standard deviation.

RESULTS AND DISCUSSION

Grain yield in average across the treatments and Type I resistance in Fusarium inoculated treatments.

Table 1. The analysis of variance for grain yield

Source of variability	DF	MS
Year (Y)	1	22819***
Treatment (T)	6	929**
Variety (V)	11	1626***
Y*T	6	831*
Y*V	11	142
T*V	66	18
Y*T*V	66	23
Error	168	319

***, **, * = significant at $P < 0.001$, 0.01 and 0.05, respectively;
DF - Degrees of freedom, MS - mean square.

The analysis of variance (ANOVA) showed significant effects ($p < 0.001$) of year and variety for the grain yield, while treatment and interaction year by treatment demonstrated lower significant effects ($p < 0.01$ and 0.05, respectively). The wheat grain yield, despite the significant effect of years, treatments and varieties depended on the interaction of years and treatments, which did not interact with varieties (Table 1).

Table 2. Average grain yield across treatments in average

Year	Average grain yield (dt ha^{-1}) across treatments in average											
	Bećar	Brko	El Nino	Garavuša	Grofica	Katarina	Kraljica	Silvija	Sofru	Tata Mata	Tika Taka	Vulkan
2019/2020	99.75b	101.21b	88.47b	93.80b	85.15b	84.74b	95.86b	80.54b	106.50b	89.25b	103.60b	92.58b
2020/2021	123.84a	130.08a	110.19a	114.42a	109.93a	107.95a	118.89a	111.74a	128.71a	106.06a	121.66a	113.03a

Average grain yield difference due to fungicide application or/and *Fusarium* inoculation across all varieties varied among treatments and years, where in vegetative year 2019/2020 there was significantly lower grain yield in average across all treatments together (Table 2). The lowest grain yield decrease recorded in 2020, compared to 2021, was in variety Tika Taka (14.85%), while the highest one was recorded in Silvija (27.92%). The average wheat yields in the studied years were variable and ranged from 106.06 dt ha^{-1} (Tata Mata) to 130.08 dt ha^{-1} (Brko) in 2021, and from 80.54 dt ha^{-1} (Silvija) to 106.50 dt ha^{-1} (Sofru) in 2020.

AUDPC for type I resistance was considerably higher (= higher susceptibility) in 2020 than in the year 2021 while the lowest range and less variable scores were observed in VI treatment in both years. The VII treatment was the most variable in terms of FHB incidence contrarily to the VI treatment (Figures 2a and 2b). In treatment VI the highest AUDPC for Type I resistance had variety Bećar (237.53 AUDPC) at the second year of investigation, being significantly different from the lowest AUDPC units of Silvija and Katarina from the second year of investigation (22.49 and 40.01 AUDPC) and Bećar from the first year of investigation (34.99 AUDPC). In treatment VII the highest AUDPC for Type I resistance had Sofru and Tika Taka (541.68 and 514.99 AUDPC) at the first year of investigation, while the lowest one was recorded in Silvija at the second year of investigation (77.52 AUDPC).

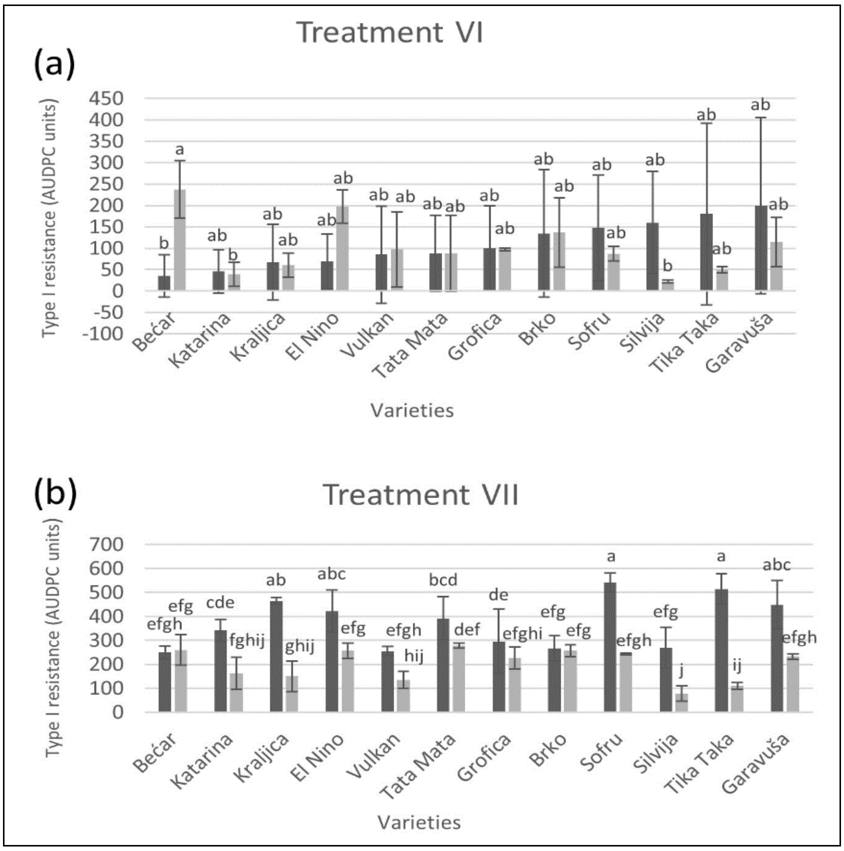

Figure 2. Type I resistance in the treatment VI (fungicide + *Fusarium*) (a) and in the treatment VII (*Fusarium*) (b) in two growing season (2019/2020 and 2020/2021)

Grain yield across years, treatments and varieties

Variety Bećar had the highest grain yield in the second year in III treatment where fungicide in the tillering was applied. This grain yield of 132.81 dt ha^{-1} was significantly different from the grain yield in the first year of investigation in VI, IV, and VII treatments with grain yields of 94.69, 88.80 and 86.57 dt ha^{-1}, respectively. The lowest yield in the second year was recorded in VI and VII treatments (116.93 and 119.33 dt ha^{-1}) which was at the same significant level with the highest grain yield in the first year in V treatment (113.26 dt ha^{-1}) (Figure 3a).

The lowest grain yield for variety Brko was obtained in the first year of investigation in VII, IV, III and VI treatments (80.85, 88.34, 97.70 and 100.65 dt ha^{-1}, respectively) which was significantly different from treatments IV, V and III form the second year of investigation with grain yields of 135.22, 141.94 and 142.71 dt ha^{-1}, respectively). In the first year the highest yield was obtained in V treatment (117.31 dt ha^{-1}), while the lowest yield at the second year was obtained in VII treatment (117.19 dt ha^{-1}) but both of those were at the same significant level (Figure 3b).

Variety El Nino had the highest yield in the second year of investigation in III treatment (125.83 dt ha^{-1}), being significantly different from the grain yield from treatments VII, VI, IV and III in the first year of investigation (90.30, 88.99, 71.38 and 64.22

dt ha^{-1}, respectively) and from the treatment VII in the second year (94.48 dt ha^{-1}). The grain yield of 114.14 dt ha^{-1} was the highest in V treatment in the first year of investigation and in the second year the lowest yield was obtained in VII treatment (94.48 dt ha^{-1}) (Figure 3c).

The highest grain yield for variety Garavuša was obtained in the second year of investigation in III treatment (127.44 dt ha^{-1}) which was significantly different from the grain yield from III, VI, IV and VII treatments in the first year of investigation (91.11, 89.23, 85.04 and 74.88 dt ha^{-1}, respectively). At the same significant level was the highest grain yield from the first year of investigation in V treatment (110.46 dt ha^{-1}) and the lowest yield from VII treatment at the second year (102.20 dt ha^{-1}) (Figure 3d).

Variety Grofica had the lowest grain yield in the first year of investigation in treatments VII, IV, VI and III (64.77, 68.14, 81.68 and 86.08 dt ha^{-1}, respectively) and in the second year of investigation in VII treatment (90.04 dt ha^{-1}), being significantly different from the grain yield in V, I and III treatments from the second year (115.95, 117.35 and 120.71 dt ha^{-1}, respectively). The lowest yield in the second year was obtained in VII treatment (90.03 dt ha^{-1}) thus being at the same significant level with the grain yield from I and V treatments from the first year (100.45 and 99.37 dt ha^{-1}) (Figure 3e).

The highest grain yield in variety Katarina was obtained in the second year of investigation in III, V and IV treatments (119.05, 116.05 and 114.53 dt ha^{-1}, respectively) which was significantly different from the grain yield from treatments I, III, II, IV and VII from the first year of investigation (90.43, 86.97, 86.47, 76.97 and 65.43 dt ha^{-1}, respectively). The highest grain yield in the first year was obtained in V treatment (96.24 dt ha^{-1}) but at the same significance as the grain yield from VI and VII treatments in the second year (96.24 and 96.83 dt ha^{-1}) (Figure 3f).

Variety Kraljica had the highest grain yield in III, V, and IV treatments from the second year of investigation which was significantly different from the grain yield from the first year of investigation in VII and IV treatments (85.54 and 82.10 dt ha^{-1}). In the first year the highest grain yield was recorded in V and I treatments (117.97 and 108.18 dt ha^{-1}), being at the same significance with the lowest grain yield in the second year in VI, II and VII treatments (106.98, 112.62 and 115.09 dt ha^{-1}, respectively) (Figure 4a).

In treatments III, I and V in the second year of investigation variety Silvija had the highest grain yields (121.75, 120.45 and 116.53 dt ha^{-1}, respectively), compared to significantly different grain yield at treatments VI, VII and IV from the first year of investigation (80.75, 65.28 and 61.32 dt ha^{-1}, respectively). The highest grain yield was obtained in the first year in I, II and V treatments (93.50, 90.23 and 89.54 dt ha^{-1}, respectively), while the lowest grain yield was obtained in the second year in VII and VI treatments (101.31 and 103.16 dt ha^{-1}) (Figure 4b).

Sofru had the highest grain yield in the second year of investigation in III treatment (142.92 dt ha^{-1}), being significantly different from the grain yield in treatments IV, VI, and VII from the first year (105.41, 101.86 and 61.17 dt ha^{-1}, respectively). At the same significance were the highest grain yield from the first year in V treatment (131.68 dt ha^{-1}) and the lowest grain yield in the second year from VII and VI treatments (108.86 and 119.66 dt ha^{-1}) (Figure 4c).

The highest grain yield for variety Tata Mata was obtained in treatments III, V and I in the second year of investigation (119.59, 109.18 and 108.82 dt ha^{-1}, respectively), which was significantly different from the grain yield at VII and IV treatments from the first year of investigation (73.72 and 73.48 dt ha^{-1}). Tata Mata had the highest yield at the first year in V treatment (104.51 dt ha^{-1}), while the lowest grain yield was in VII treatment at the second year (93.63 dt ha^{-1}) (Figure 4d).

Variety Tika Taka had the lowest grain yield in treatments VII and IV in the first year of investigation (71.88 and 99.48 dt ha^{-1}) which was significantly different from the grain yield in treatment III in the first year of investigation and treatments II, IV, I, V and

III in the second year of investigation (119.09, 122.63, 122.64, 123.97, 125.70 and 130.15 dt ha^{-1}, respectively). In the first year the highest grain yield was recorded in III treatment (119.09 dt ha^{-1}), but at the same significant level as the lowest yield at the second year in VII treatment (109.10 dt ha^{-1}) (Figure 4e).

The highest grain yield in variety Vulkan was obtained in the second year of investigation in V, III, II, I and IV treatments (119.53, 117.74, 117.68, 114.07 and 111.63 dt ha^{-1}, respectively) which was significantly different from the grain yield in treatment IV from the first year of investigation (82.27 dt ha^{-1}). In I and V treatments there was the highest grain yield at the first year (106.36 and 99.48 dt ha^{-1}), but at the same significance as grain yield at the second year in VI treatment (101.84 dt ha^{-1}) (Figure 4f).

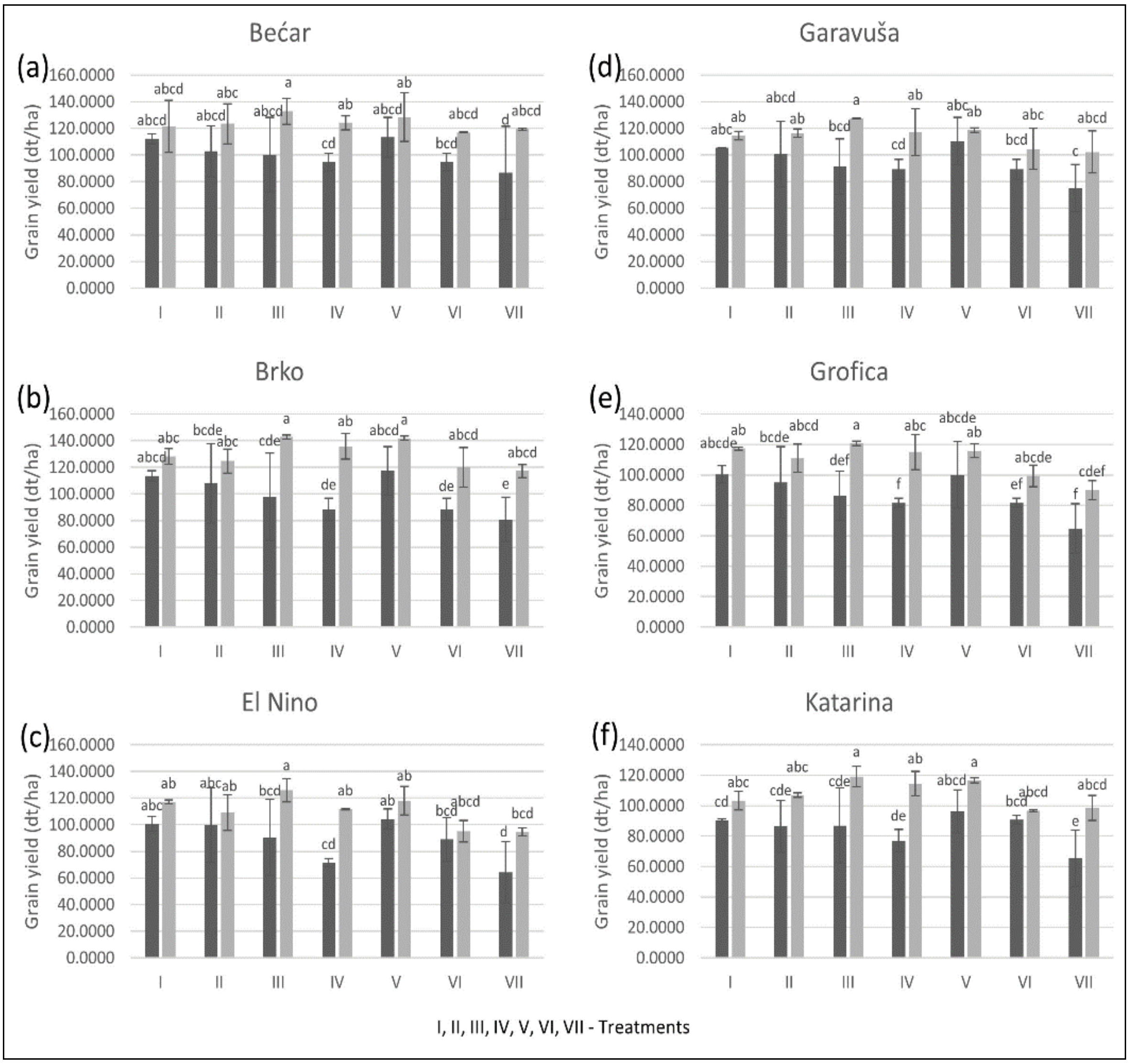

Figure 3. The grain yield (dt ha^{-1}) in seven treatments
I - seeding rate 330 g m^{-2}, II - seeding rate 600 g m^{-2}, III - fungicide application in tillering stage, IV - fungicide application in tillering + heading stages, V - fungicide application in heading stage, VI - fungicide application in heading stage + *Fusarium* inoculation, VII - *Fusarium* inoculation) in two growing season (1 - 2019/2020 and 2 - 2020/2021) for varieties Bećar (a), Brko (b), El Nino (c), Garavuša (d), Grofica (e) and Katarina (f)

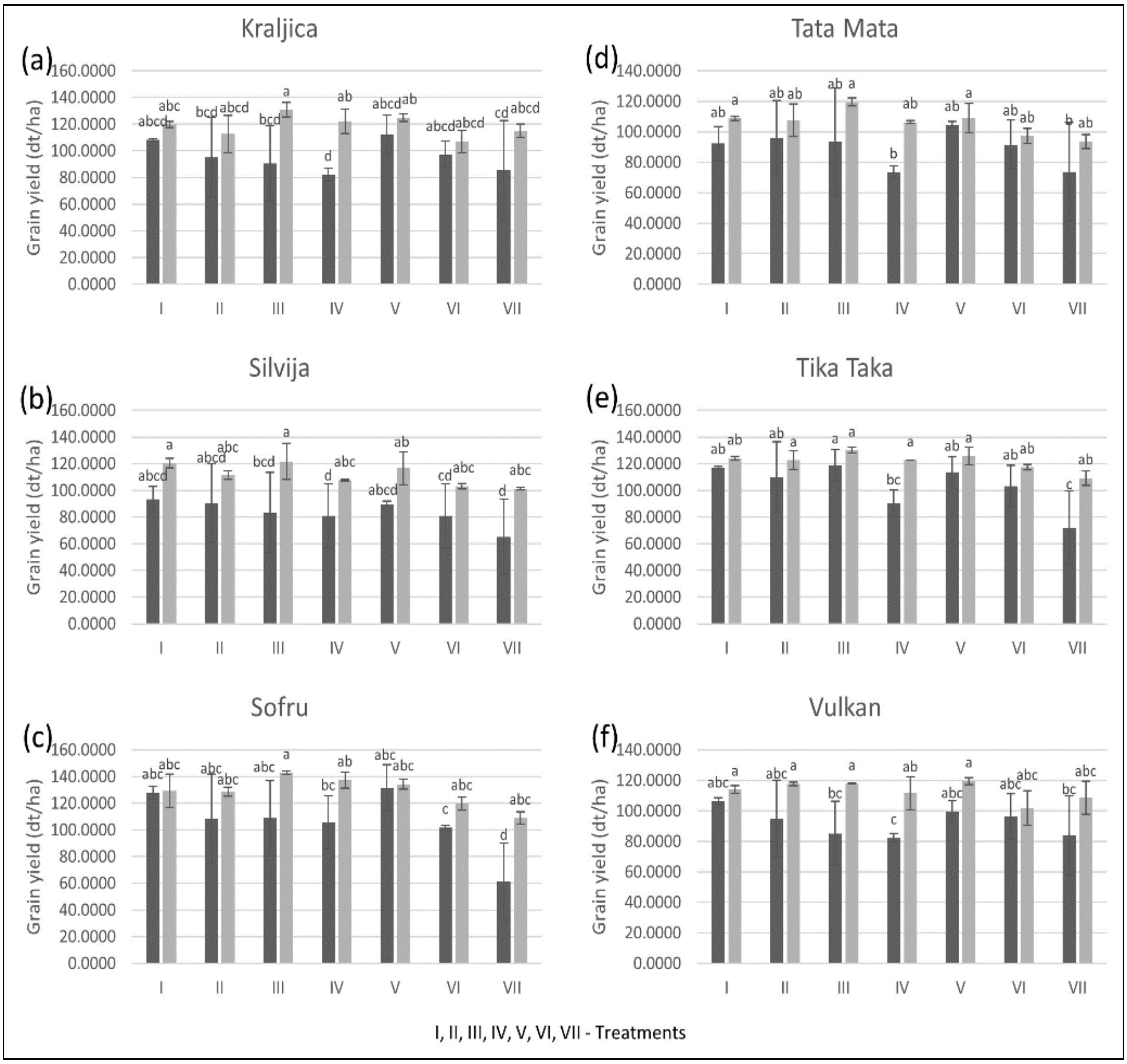

Figure 4. The grain yield (dt ha^{-1}) in seven treatments
I - seeding rate 330 g m^{-2}, II - seeding rate 600 g m^{-2}, III - fungicide application in tillering stage, IV - fungicide application in tillering + heading stages, V - fungicide application in heading stage, VI - fungicide application in heading stage + *Fusarium* inoculation, VII - *Fusarium* inoculation) in two growing season (1 - 2019/2020 and 2 - 2020/2021) for varieties Kraljica (a), Silvija (b), Sofru (c), Tata Mata (d), Tika Taka (e) and Vulkan (f)

This report summarizes the results of testing twelve winter wheat varieties varying in FHB disease susceptibility in two treatments with no fungicide in two seeding rates, in four fungicide application treatments (treatment in tillering stage; treatment in tillering + heading stage; treatment in heading stage; and treatment in heading stage + *Fusarium* inoculation), and only *Fusarium* inoculated treatment. Research has documented the potential to control FHB through a management system that integrates variety selection and fungicide application at different growth stages. As grain yield is a function of interaction amongst various yield components affected differently by the growing conditions and crop management practices (Cheema et al., 2010) the data presented in this research shows that the interactions of the studied experimental factors (year and treatment) had significant effects on the grain yield of wheat. First, the weather conditions from the 2019/2020 and 2020/2021 growing seasons differed considerably. Year 2021 was considered a good year in terms of the grain yield in the region of Croatia. In 2021 plants had enough water whereas the warm dry conditions that followed during flowering from late May to mid-June prevented FHB during this growing season. Similar results for the grain yield during similar weather conditions were obtained by Mueller et al.

(2017). On the other hand, 2020 was completely different year than 2021. High precipitation in May and June was favorable for FHB infection. Similarly, it was obtained by Sassenrath et al. (2021) when very wet spring in 2020 resulted in FHB infection of wheat. Due to low precipitation in May and June 2021, there was a little disease pressure. However, a rainy June in 2020 allowed higher disease pressure after flowering with FHB as the main diseases observed. High humidity and rainfall during May and June 2020 resulted in high fungal infection with FHB rate in wheat. Vegetative year, as a main effect or interacting with fungicide or *Fusarium* treatment, had a significant effect on the grain yield. This is likely due to the considerable differences in environmental conditions between the two growing years, as discussed. Similarly, it was reported in the investigation of Martínez et al. (2012). Despite the significant effect of growing year, similar trends of grain yields were observed in both growing seasons under each fungicide treatment.

FHB is the most widespread wheat disease worldwide which results in total or partial head premature senescence with a consequent reduction in grain yields and quality (Champeil et al., 2004). But many other wheat diseases which are of particular importance for flag leaf during grain filling could occurred thus reducing grain yield, due to a reduction of photosynthetic activity (Richards, 2000). Besides the most important preventive measures for wheat diseases by using crop rotation or usage of resistant varieties (Tóth et al., 2008) in the vegetative seasons conductive to fungal diseases, direct control through the use of fungicide application is necessary (McMullen et al., 2008). FHB infection in wheat can occur anytime the head has emerged until the start of senescence. FHB impacts on grain yield and quality can be reduced through fungicide application at head timing (MacLean et al. 2018). Therefore, wheat heads are the most susceptible to FHB infection at anthesis but infection can occur up to the soft dough stage (Lacey et al., 1999). To investigate the relationship between variety susceptibility and fungicide treatment in the heading stage, the FHB resistance/susceptibility of varieties varied considerably in treatments VI (fungicide application + *Fusarium* inoculation) and VII (only *Fusarium* inoculation). Then, the difference in Type I resistance without fungicide treatment (treatment VII) was significantly under the influence of the growing season, while treatment VI with fungicide + *Fusarium* inoculation influenced ranking of wheat varieties in Type I resistance, where growing season had much less influence, except for variety Bećar which showed significant difference in resistance in those two years. Czaban et al. (2015) suggested that winter wheat kernel infection by *Fusarium* spp. depends primarily by weather conditions and then by the wheat variety. Covarelli et al. (2015) concluded that infections increase was closely related to the amount of precipitations during wheat anthesis. For example, in Italy, FHB as well as DON contamination have been reported in several regions with different intensity depending on the year, cultivation area and durum wheat variety (Pancaldi et al., 2010). In the current research, conversely, the lowest FHB incidence was in growing season with the lower precipitation in June.

According to Sicher (1993) the top three leaves on a stem, especially the flag leaf, absorb most irradiation light, and were the primary source of carbohydrate production. But despite of that, the flag leaf of the wheat plant, the last leaf to emerge, is responsible for 50% of the effective leaf area that contributes to grain fill and thus could be considered to be the first key organ contributing to higher grain yields in wheat (Liu et al., 2018). For this reason, protecting the flag leaf is imperative to getting maximum crop quality and yield. Unless that a disease like rusts showed up at the flag leaves, fungicide application could be skipped at the flag leaf stage and conduct it at the heading stage. While both FHB risk and foliar leaf disease at heading may warrant a fungicide application at anthesis. The goal of fungicides is to protect the plant's photosynthetic area, to reduce impacts of FHB, or a combination of both. Therefore, spraying at the heading stage will protect grain yield from both leaf spotting disease and FHB. According to Wiersma and Motteberg (2005), application of fungicide at heading stage provided equal or better yield results than a flag leaf timing fungicide. The application of fungicides to wheat from heading to anthesis could be an essential practice to reduce yield loss due to fungal diseases attacking heads and flag leaves (Mesterházy et al., 2003). In the current research, grain yield response as a result of fungicide application was not the same across wheat varieties. The highest grain yields were obtained in varieties Brko, Bećar, Tika Taka and Sofru across all treatments (except in VII treatment in both years and in VI treatment in the first year of investigation). The highest yields in the first year in *Fusarium* inoculated (VII) treatment had varieties Bećar, Kraljica, Vulkan and Brko, whereas in the second year the highest yields had Bećar, Brko, Kraljica and Tika Taka. It could be observed that variety Sofru had the highest AUDPC for FHB type I resistance (the most susceptible to initial infection) in VII treatment, and therefore its grain yield decreased considerably in *Fusarium* treated plots without usage of fungicides. For instance, Wegulo et al. (2011) reported that fungicides reduced FHB and DON more in moderately resistant varieties than in a susceptible variety. In 2020 eight varieties had the lowest grain yield response in treatment VII where only *Fusarium* inoculation was applied in the heading stage, with the exception of four varieties (Kraljica, Silvija, Tata Mata and Vulkan), in which field plots receiving fungicides at tillering and heading stages together gave the lowest yields. The highest yields in 2020 were achieved in treatment V where fungicide was applied at the heading stage. Similarly, it was obtained by Asif et al. (2021) where the most economically beneficial practices were fungicide applications at flag leaf timing (BBCH 39-45) or FHB timing (BBCH 61-63) when environmental conditions were conducive for disease development. For this reason, protecting the heads during the seasons subjected to FHB disease is critical to achieving grain yield goals. But must be taken into account, that maize pre-crop in 2020 also could have influenced FHB infections. Dill-Macky and Jones (2000) revealed that previous crop residue and tillage practices differentially affected the incidence and severity of FHB disease. Implementing crop rotation can improve overall productive capacity of fields by reducing FHB pressure. This may be especially important in high rainfall season. In the study of Szczepaniak et al. (2022) the wheat-grain yield depended significantly on grain density where the relative increase in grain density in fungicide treatment, compared without fungicide application, was approximately 18%.

The ideal treatment in 2021 had the highest grain yield response due to fungicide application in tillering stage of plant growth in eleven winter wheat varieties, while in variety Vulkan in treatment V with both fungicide applications in tillering and heading stages together gave the highest grain yield. If conditions are favorable for disease development early in the season, it is beneficial to apply a fungicide for early season disease suppression and follow up with a second application at anthesis. The Asif et al. (2021) included fungicide treatments at herbicide timing (BBCH 22-23) around tillering stage but these early timings did not increase yields. According to Dimmock and Gooding (2002) fungicides provide protection against fungal pathogens, especially on the flag leaf, the main contributor to grain yield whereas the majority of producers apply fungicides at flowering for the management of FHB. Treatments without usage of fungicides with different seeding densities were higher in the second year (2021), but at both investigated years there were no significant differences between I and II treatments with lower and higher seed densities. In the current study, each increment in seeding rate did not increase grain yield. Opposite to that, Schaafsma and Tamburic-Ilincic (2007) reported that the number of spikes per m^2 and yield increased with increased seeding densities. Our study suggests that environmental conditions at local level are determinant factors in controlling potential FHB outbreak where results from fungicide efficacy trials may vary with year and variety. Year was the factor that leads to the greatest improvement in yields for all varieties and all treatments, potentially due to a genetic potential of variety. The wet conditions of 2020 in May and June resulted in higher FHB pressure, but dry conditions in 2021 in May and June reduced FHB contamination. Fungicide application in tillering stage had a larger impact on the grain yield improvement in 2021 than fungicide applications in the heading stage in 2020. The profitability of fungicide application is influenced by susceptibility of the varieties, disease pressure as a result of weather conditions.

CONCLUSIONS

Management of FHB requires an integrated approach incorporating the use of good pre-crop, fungicides, and variety resistance. Selection of FHB resistant wheat varieties is one of the key approaches to improve wheat grain yields and reduce losses due to fungal infections, especially in the growing season with the high rainfall. Important influence in the incidence of FHB infection could have crop rotation. The highest FHB incidence was observed in the growing season where maize was used as a pre-crop.

One finding is that a great opportunity to optimize the grain yield potential of certain wheat varieties is by applying a fungicide at tillering and heading stages, whether disease pressure is present or not, to protect plants in the most sensitive period for creation of grain yield. Therefore, at least two chemical controls provide great opportunity for controlling wheat diseases. The key to making a successful fungicide application at tillering or anthesis is to begin spraying preventatively as soon as climatic conditions are favorable for disease development. If spraying is provided until a disease has already manifested, irreversible loss of yield potential could occur.

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REFERENCES

Asif, M., Strydhorst, S., Strelkov, S.E., Terry, A., Harding, M.W., Feng, J., Yang, R.C., 2021. *Evaluation of disease, yield and economics associated with fungicide timing in Canadian Western Red Spring Wheat.* Can. J. Plant Sci., 101: 680-697.

Beres, B.L., Brûlé-Babel, A.L., Ye, Z., Graf, R.J., Turkington, T.K., Harding, M.W., Kutcher, H.R., Hooker, D.C., 2018. *Exploring Genotype × Environment × Management Synergies to Manage Fusarium Head Blight in Wheat*. Can. J. Plant Pathol., 40: 179-188.

Buerstmayr, H., Lemmens, M., Fedak, G., Ruckenbauer, P., 1999. *Back-cross reciprocal monosomic analysis of Fusarium head blight resistance in wheat (Triticum aestivum L.)*. Theor. Appl. Genet., 98: 76-85.

Champeil, A., Dore, T., Fourbet, J.F., 2004. *Fusarium head blight: epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains*. Plant Sci., 166: 1389-1415.

Cheema, M., Farhad, W., Saleem, M., Khan, H., Munir, A., Wahid, M., Rasul, F., Hammad, H., Cheema, M., Farhad, W., 2010. *Nitrogen management strategies for sustainable maize production*. Crop Environment, 1: 49-52.

Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C., Ferrer, E., Mañes, J., 2015. *Fusarium species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy*. J. Sci. Food Agric., 95: 541-551.

Czaban, J., Wróblewska, B., Sułek, A., Mikos, M., Boguszewska, E., Podolska, G., Nieróbca, A., 2015. *Colonisation of winter wheat grain by Fusarium spp. and mycotoxin content as dependent on a wheat variety, crop rotation, a* *crop management system and weather conditions*. Food Addit. Contam., 32: 874-910.

Dahl, B., and Wilson, W.W., 2018. *Risk Premiums Due to Fusarium Head Blight (FHB) in Wheat and Barley*. Agric. Syst., 162: 145-153.

Dill-Macky, R., and Jones, R.K., 2000. *The effect of previous crop residues and tillage on Fusarium head blight of wheat*. Plant Dis., 84: 71-76.

Dimmock, J.P.R.E., and Gooding, M.J., 2002. *The effects of fungicides on Hagberg falling number and blackpoint in winter wheat*. Crop Prot., 21: 475-487.

ElDoliefy, A.E.F.A., Kumar, A., Anderson, J.A., Glover, K.D., Mamidi, S., Elias, E.M., Seetan, R., Alamri, M.S., Kianian, S.F., Sapkota, S., Green, A., Mergoum, M., 2020. *Genetic dissection of Fusarium head blight resistance in spring wheat cv. 'Glenn.'*. Euphytica, 216: 71.

Kriss, A.B., Paul, P.A., Madden, L.V., 2010. *Relationship between Yearly Fluctuations in Fusarium Head Blight Intensity and Environmental Variables: A Window-Pane Analysis*. Phytopathology, 100: 784-797.

Kriss, A.B., Paul, P.A., Xu, X., Nicholson, P., Doohan, F.M., Hornok, L., Rietini, A., Edwards, S.G., Madden, L.V., 2012. *Quantification of the relationship between the environment and Fusarium head blight, Fusarium pathogen density, and mycotoxins in winter wheat in Europe*. Eur. J. Plant Pathol., 133: 975-993.

Lacey, J., Bateman, G.L., Mirocha, C.J., 1999. *Effects of Infection Time and Moisture on Development of Ear Blight and Deoxynivalenol Production by Fusarium Spp. in Wheat*. Ann. Appl. Biol., 134: 277-283.

Liu, J., Feng, B., Xu, Z., Fan, X., Jiang, F., Jin, X., Cao, J., Wang, F., Liu, Q., Yang, L., Wang, T., 2018. *Identification of QTL for flag leaf length in common wheat and their pleiotropic effects*. Mol. Breed., 38: 1-11.

MacLean, D.E., Lobo, J.M., Coles, K., Harding, M.W., May, W.E., Peng, G., Turkington, T.K., and Kutcher, H.R., 2018. *Fungicide application at anthesis of wheat provides effective control of leaf spotting diseases in western Canada*. Crop Prot., 112: 343-349.

Martínez, M., Moschini, R., Barreto, D., Comerio, R., 2012. *Effect of Environment on Fusarium Head Blight Intensity and Deoxynivalenol Content in Wheat Grains: Development of a Forecasting System*. Cereal Res. Commun., 40: 74-84.

McMullen, M., Halley, S., Schatz, B., Meyer, S., Jordahl, J., Ransom, J., 2008. *Integrated strategies for Fusarium head blight management in the United States*. Cereal Res. Commun., 36: 563-568.

Mesterhazy, A., Bartok, T., Lamper, C., 2003. *Influence of wheat cultivar, species of Fusarium, and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight*. Plant Dis., 87: 1107-1115.

Mickky, B., Aldesuquy, H., Elnajar, M., 2020. *Effect of drought on yield of ten wheat cultivars linked with their flag leaf water status, fatty acid profile and shoot vigor at heading Physiol*. Mol. Biol. Plants, 26: 1111-1117.

Mueller, N., Glewen, K., Williams, T., Hay, P., Lesoing, G., Pryor, R., 2017. *Recap of 2017 eastern Nebraska winter wheat crop*. In: Institute of Agriculture and Natureal Resources - University of Nebraska-Lincoln. Available from: https://cropwatch.unl.edu/2017/recap-2016-17-wheat-season-eastern-nebraska (Last accessed: 11.02.2022).

Okorski, A., Milewska, A., Pszczółkowska, A., Karpiesiuk, K., Kozera, W., Dąbrowska, J.A., Radwińska, J., 2022. *Prevalence of Fusarium fungi and Deoxynivalenol Levels in Winter Wheat Grain in Different Climatic Regions of Poland*. Toxins, 14(2): 102.

Pancaldi, D., Tonti, S., Prodi, A., Salomoni, D., Dal Prà, M., Nipoti, P., Alberti, I., Pisi, A., 2010. *Survey of the main causal agents of Fusarium head blight of durum wheat around Bologna, northern Italy*. Phytopathol. Mediterr., 49: 258-266.

Parry, D.W., Jenkinson, P., McLeod, L., 1995. *Fusarium ear blight (scab) in small grain cereals-a review*. Plant Pathol., 44: 207-238.

Pietrusińska, A., and Tratwal, A., 2020. *Characteristics of powdery mildew and its importance for wheat grown in Poland*. Plant Protect. Sci., 56: 141-153.

Richards, R.A., 2000. *Selectable traits to increase crop photosynthesis and yield of grain crops*. J. Exp. Bot., 51: 447-458.

Rowlandson, T., Gleason, M., Sentelhas, P., Gilespie, T., Thomas, C., Hornbuckle, B., 2015. *Reconsidering leaf wetness duration determination for plant disease management*. Plant Dis., 99: 310-319.

Sassenrath, G.F., M. Knapp, X. Lin, 2021. *Southeast Kansas Weather Summary - 2020*. Kansas Agricultural Experiment Station Research Reports, 7. https://newprairiepress.org/cgi/viewcontent.cgi?article=8053&context=kaesrr (Last accessed: 26.01.2022).

Scala, V., Aureli, G., Cesarano, G., Incerti, G., Fanelli, C., Scala, F., Reverberi, M., Bonanomi, G., 2016. *Climate, Soil Management, and Cultivar Affect Fusarium Head Blight Incidence and Deoxynivalenol Accumulation in Durum Wheat of Southern Italy*. Front. Microbiol., 7.

Schaafsma, A.W., and Tamburic-Ilincic, L., 2007. *Effect of Seeding Rate and Seed Treatment Fungicides on Agronomic Performance, Fusarium Head Blight Symptoms, and DON Accumulation in Two Winter Wheats*. Plant Dis., 89: 1109-1113.

Schauberger, B., Ben-Ari, T., Makowski, D., Kato, T., Kato, H., Ciais, P., 2018. *Yield trends, variability and stagnation analysis of major crops in France over more than a century*. Sci. Rep., 8: 16865.

Sicher, R.C., 1993. *Assimilate partitioning within leaves of small grain cereals*. In: Abrol, Y.P., Mohanty, P., Govindjee (eds.), Photosynthesis:

Photoreactions to Plant Productivity. Springer, Netherlands: 351-360.

Szczepaniak, W., Nowicki, B., Bełka, D., Kazimierowicz, A., Kulwicki, M., Grzebisz, W., 2022. *Effect of foliar application of micronutrients and fungicides on the nitrogen use efficiency in winter wheat*. Agronomy, 12: 257.

Torriani, S.F.F., Melichar, J.P.E., Mills, C., Pain, N., Sierotzki, H., Courbot, M., 2015. *Zymoseptoria tritici: a major threat to wheat production, integrated approaches to control*. Fungal Genet. Biol., 79: 8-12.

Tóth, B., Kaszonyi, G., Bartok, T., Varga, J., Mesterhazy, A., 2008. *Common resistance of wheat to members of the Fusarium graminearum species complex and F. culmorum*. Plant Breed., 127: 1-8.

Thompson, N.M., Epplin, F.M., Edwards, J.T., Hunger, R.M., 2014. *Economics of foliar fungicides for hard red winter wheat in the USA southern Great Plains*. Crop Prot., 59: 1-6.

Zadoks, J.C., Chang, T.T., Konzac, F.C., 1974. *A decimal code for the growth stages of cereals*. Weed Res., 14: 415-421.

Wegulo, S.N., Zwingman, M.V., Breathnach, J.A., Baenziger, P.S., 2011. *Economic returns from fungicide application to control foliar fungal diseases in winter wheat*. Crop Prot., 30: 685-692.

Wiersma, J.J., and Motteberg, C.D., 2005. *Evaluation of five fungicide application timings for control of leaf-spot diseases and fusarium head blight in hard red spring wheat*. Can. J. Plant Pathol., 27: 25-37.

Wiik, L., and Ewaldz, T., 2009. *Impact of temperature and precipitation on yield and plant diseases of winter wheat in southern Sweden 1983-2007*. Crop Prot., 28: 952-962.